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TITLE: microRNA in Cerebral Spinal Fluid as Biomarkers of Alzheimer's Disease Risk After Brain Injury

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14. ABSTRACT

A history of TBI increases the odds of developing AD by 2.5 times in the general population, and 4-6 times for military veterans. Although significant associations between mTBI and risk of AD have been observed, the precise mechanism by which TBI might lead to AD and/or AD-related symptoms are not yet understood. Histologically, AD is characterized by amyloid- and neurofibrillary protein aggregates, suggesting a loss of protein processing is a key feature of AD. MiRNAs are small non- coding RNA that regulate mRNA transcription, and may be a significant cause of protein dysregulation.
In a study recently accepted for publication in the Journal of Alzheimer's Disease (Lusardi et al, JAD 16-0835 In Press), we show that miRNA can be measured in CSF from living human donors and there are distinct differences in donors with confirmed AD from age- and sex-matched control donors. Further, we demonstrate that panels of 3 - 4 miRNA predict AD as well as ApoE status, and in combination with ApoE improve classification performance. These studies show that changes in CSF miRNA can be reliably detected by qPCR, and will provide additional data in understanding the longitudinal changes in CSF miRNA expression changes associated with AD.

15. SUBJECT TERMS

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1. Introduction

A history of TBI increases the odds of developing AD by 2.5 times in the general population, and 4-6 times for military veterans, and accelerates cognitive decline. Although significant associations have been observed, the precise mechanism by which TBI might lead to AD and/or AD-related symptoms are not yet understood. Protein biomarkers related to known AD pathologies and measured in CSF are very sensitive markers of AD, but they lack specificity, often showing up in individuals with no clinical signs of AD. It is not clear whether these protein biomarkers reflect necessary, but insufficient, processes leading to AD, or whether they reflect an early disease stage that, given enough time, will lead to AD. Histologically, AD is characterized by amyloid- and neurofibrillary protein aggregates, suggesting a loss of protein processing is a key feature of AD. MiRNAs are a recently discovered class of small non- coding RNA that regulate mRNA transcription, and may be a significant cause of protein dysregulation. Our investigative team has generated preliminary data showing that the miRNA distribution in CSF is altered in civilians with Alzheimer's disease (AD). Specifically, the signature of miRNA expression in AD is a decrease in abundance or the absence of a subset of miRNAs, which is consistent with the signature pathology of protein over expression and accumulation in AD. Further, when considering the TBI history of our subjects, we find that those with a history of TBI are over-represented in our AD group, and we find a specific group of miRNAs regulated in this population. We hypothesize that TBI induces an alteration in CSF miRNA patterns that reflect the initial molecular responses to brain injury that precede, and likely drive, changes in protein expression that lead to the development of AD. We have additional preliminary data showing altered protein biomarkers in deployed veterans with a history of TBI, and additional protein biomarkers specific for deployment, regardless of TBI history.

2. Keywords

Mild traumatic brain injury (mTBI), Alzheimer's disease (AD), miRNA, cerebral spinal fluid (CSF), biomarker, deployment, blast injury

3. Accomplishments

What were the major goals of the project?

Specific Aim 1: CSF miRNA measurement from mTBI and Controls

Major Task 1: Regulatory Approval

Major Task 2: CSF miRNA Measurement from mTBI and Controls

Major Task 3: Identify Candidate Biomarkers

Specific Aim 2: Identify miRNA as Biomarkers of mTBI

Major Task 4: FITBIR Data Sharing

Major Task 5: Biostatistical Modeling

Major Task 6: Biomarker Candidate Verification

Specific Aim 3: AD Pathway-Directed Bioinformatics Evaluation of mTBI-regulated miRNA

Major Task 7: Bioinformatics Analyses

Major Task 8: Manuscript Preparation

What was accomplished under these goals?

Major Task 1: Regulatory Approval has been obtained from OHSU and SIBCR Institutional Review Boards and from HRPO (Log Number A-18952).

Major Task 2: Standardized CSF miRNA qPCR, analysis, and quality control protocols.

Major Task 4: We have established FITBIR pseudoGUIDs for all of the subjects in the project.

Tasks 3, 5, 6: Due to the regulatory delays, we have nothing to report for these tasks. Despite the delays, we do not anticipate any technical difficulties or budget impacts in completing these tasks.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

During the next quarter, we will begin the project specific CSF miRNA assay (Task 2); personnel and lab resources have been assigned to facilitate assay completion at a faster rate than originally proposed (40/quarter). Assay materials from single lots have been purchased to minimize lot-specific processing effects. Samples from each experimental group (deployed + mTBI, deployed with no TBI history, community controls) will be randomized for analysis to minimize batch effects. QC analysis will be performed as data becomes available. Preliminary individual biomarker candidates (Task 3) will be developed once 40 assays are available, then refined as the data set grows by at least 40 additional samples.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. Changes/Problems

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

We experienced significant delays in obtaining regulatory approval for these studies, which have impacted our schedule. We have discussed this delay with our Scientific Officer, Dr. Stephen J. Grate.

Changes that had a significant impact on expenditures

We do not anticipate that the regulatory delays will impact expenditures for this project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

6. Products

• Publications, conference papers, and presentations

Our related work showing that CSF miRNA can discriminate AD from age- and sex-matched control donors has been accepted for publication by the Journal of Alzheimer's Disease (in press). Combinations of miRNA improved sensitivity and specificity of biomarker performance, and in combination with ApoE genotype further improved classification. This work utilized the same measurement platform (TLDA qPCR) as the present study, and not only verifies the method, but provides important information on the state of CSF miRNA in donors with confirmed AD. We will utilize this information in the bioinformatics analysis to document longitudinal changes in CSF miRNA and their implications for biological mechanisms underlying AD pathology.

• Website(s) or other Internet site(s)

Nothing to Report.

• Technologies or techniques

Nothing to Report.

• Inventions, patent applications, and/or licenses

Nothing to Report.

• Other Products

Nothing to Report.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	**Dr. Joseph Quinn**
Project Role:	PI
Researcher Identifier (e.g., ORCID ID):	0000-0001-7305-2256
Nearest person month worked:	1
Contribution to Project:	Dr. Quinn has overseen the regulatory approval process and quarterly reporting.
Funding Support:	*See below*

Name:	**Dr. Theresa A. Lusardi**
Project Role:	Co - PI
Researcher Identifier (e.g., ORCID ID):	0000-0003-0699-5662
Nearest person month worked:	1
Contribution to Project:	Dr. Lusardi has assisted with the regulatory approval process, and negotiated the materials purchasing.
Funding Support:	*See below*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Changes to Active support for Dr. Joseph Quinn

FTE	Funding Source	Changes
0.025	NIH **R01 AT0008099**	Added
0.06	Stanford University - NIH Pacific Northwest Udall Center	Added

Dr. Theresa Lusardi has recently joined OHSU as a Senior Research Associate in the School of Medicine, Computational Biology Program. Her present funding support consists of:

FTE	Funding Source	Changes
0.25	USAMRMC W81XWH-15-1-0318	This effort replaces the LRI subaward to Dr. Lusardi
0.30	NIH UH2TR00090	Unchanged from prior reports
0.45	OHSU University Shared Resources	Added OHSU Bioinformatics Core responsibilities

What other organizations were involved as partners?

Organization Name	Seattle Institute for Biomedical and Clinical Research (SIBCR)
Location of Organization	1660 S. Columbian Way, MS S-151, Seattle, WA 98108-1532
Partner's Contribution	*Collaboration*: Provide banked CSF samples, corresponding clinical and biomarker data. Analytic support, including integration of findings resulting from this project with ongoing multimodal studies of the same participant group. Contribute to preparation of abstracts and manuscripts resulting from the research.

8. Special Reporting Requirements

None.

9. Appendices

- *MicroRNAs in Human Cerebrospinal Fluid as Biomarkers for Alzheimer's Disease,* Theresa A. Lusardi, Jay I. Phillips, Jack T. Wiedrick, Christina A. Harrington, Babett Lind, Jodi A. Lapidus, Joseph F. Quinn, Julie A. Saugstad. Journal of Alzheimer's Disease 16-0835 *In Press*